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Cellular and Molecular Mechanisms That Direct Leukocyte Traffic

Eugene C. Butcher

From the Department of Pathology, Stanford University School of Medicine, Stanford, California, and the Veterans Administration Medical Center, Palo Alto, California

The traffic of leukocytes displays exquisite specificity *in vivo*. Neutrophils selectively enter sites of acute inflammation or tissue damage. Eosinophils extravasate into sites of allergic reaction or parasitic infestation. Monocytes are recruited preferentially during subacute and granulomatous inflammation. Small lymphocytes recirculate from one lymphoid organ to another *via* continuous passage from blood, through lymphoid organs and lymph, and back to the blood. Particular subsets of lymphocytes, for example many activated lymphocytes and probably memory cells, traffic with remarkable selectivity to or through particular organs or regions of the body. The overall process of leukocyte homing to particular tissue microenvironments is clearly regulated at many levels. The first level of traffic control, however, is binding of circulating leukocytes to venular endothelium. In this lecture I will review our studies of leukocyte–endothelial cell adhesive mechanisms, and will outline our concepts of the importance of these mechanisms in the physiology of immune and inflammatory responses.

Organ-Specific Lymphocyte Interactions with High Endothelial Venules

Circulating lymphocytes recognize, bind to, and extravasate *via* specialized high endothelial venules (HEV) in lymph nodes, in mucosal lymphoid tissues such as Peyer's patches and appendix, and in sites of chronic inflammation.[1,2] We have studied lymphocyte-HEV interactions both in short-term *in vivo* homing assays, and in an elegant *ex vivo* system, developed by Judith Woodruff and her colleagues,[3] in which viable lymphocytes bind with remarkable specificity to HEV in frozen sections of lymph nodes or other tissue sites of lymphocyte homing.

One of the most exciting observations made using these assays is that lymphocytes can discriminate between HEV in different body tissues. For example, certain murine or human lymphomas, or normal lymphocyte subsets such as gut-homing mesenteric node immunoblasts, gut intraepithelial leukocytes, or lamina propria lymphoblasts, bind to HEV in mucosal lymphoid tissues but not to peripheral lymph node (PLN) HEV.[4–6] Conversely, other lymphomas or lymphoid cell lines bind selectively to PLN HEV (eg, the axillary, brachial, cervical, popliteal, or paraaortic lymph nodes in the mouse).[4,7] The mechanisms that allow such organ-specific recognition of HEV are preserved across species barriers.[8]

The ability of normal lymphocyte populations to bind PLN and mucosal HEV is precisely regulated during antigen-independent and antigen-dependent lymphocyte differentiation.[2] Although certainly an oversimplification, in general it appears that most virgin B and T lymphocytes (mature lymphocytes that have not yet encountered their cognate antigen) express homing receptors for both lymph nodes and mucosal HEV (and probably for other HEV specificities as well,[9] see below), allowing them to traffic widely. This ensures that the full repertoire of clonal lymphocyte specificities is available to respond to antigen throughout the body. Quantitative differences in HEV-binding preferences of B *versus* T lymphocytes,[10] and of T-cell subsets[11] do exist, and these may contribute to the predominance of B over T cells in mucosal lymphoid organs, of T over B cells in PLNs, and of CD4+ *versus* CD8+ T cells in PLN and in most extralymphoid inflammatory sites. It is also possible that some unique subsets of lymphocytes may leave the thymus or bone marrow already restricted in their endothelial cell (EC) interaction and homing capacity. Candidates for such populations might include distinct epithelial T-cell populations or B

Address reprint requests to Eugene C. Butcher, M.D., Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305-5324.

cells of the Ly1/Mac-1$^+$ lineage, a self-renewing B-cell subset that represents a major precursor of plasma cells in the adult,[12] and that populates extralymphoid sites preferentially.[13]

Many T and B effector cells and presumptive memory cell populations display highly selective trafficking and tissue-specific EC recognition.[2] Although it has not been possible to follow directly the conversion of individual lymphocytes from dual or multiple homing specificity to restricted homing behavior, the available information suggests that antigenic stimulation of virgin lymphocytes within lymphoid microenvironments results in reprogramming of migratory specificities. Thus a B or T lymphocyte responding to antigen within a mucosal lymphoid or extralymphoid site may be programmed to lose peripheral node-homing receptors, and to traffic selectively back to mucosal tissues. Such programming may be responsible, for example, for the selective localization of IgA plasma cell precursors to mucosal sites. The same B or T lymphocyte encountering antigen within a PLN might lose mucosal homing properties, perhaps with retention of PLN homing receptors and/or expression of novel receptors for endothelial determinants associated with extralymphoid inflammatory sites.

Highly specific lymphocyte–HEV recognition also appears to target lymphocyte homing to other tissues or organs in the body. Venules in inflamed synovium, for example in rheumatoid arthritis or Lyme disease, bind lymphocytes *via* a mechanism clearly distinct from those of Peyer's patch and PLN HEV.[9] HEV in sections of peribronchial lymph nodes[14] and in superficial dermal vessels in inflammatory dermatoses such as psoriasis[15] (N. Wu, unpublished data) also bind lymphocytes, again *via* mechanisms distinct from PLN or Peyer's patch HEV. It is possible that targetted immunosuppressive therapies could be developed based on selective inhibition of the lymphocyte and endothelial cell receptors involved.

An important question for future investigation is whether lymphocyte binding to synovial, skin, and perhaps even peribronchial LN HEV involves tissue-specific EC recognition analogous to interactions with mucosal and PLN HEV, and/or more general inflammation-associated adhesion molecules induced on EC by local inflammation and on lymphocytes by antigenic stimulation. In the latter context, it is relevant that populations of lymphocytes retrieved from inflammatory sites can migrate with remarkable efficiency to extralymphoid sites of inflammation, such as dermal sites of delayed-type hypersensitivity, consistent with the existence of leukocyte-EC recognition systems preferentially used during tissue inflammation.[16] Furthermore, activated and memory lymphocytes express elevated levels of a number of adhesion molecules, including several known to be involved in lymphocyte–endothelial interactions.[17,18]

Role of HEV Recognition in Lymphoma Metastasis

The ability to recognize and bind HEV is not only important in regulating the traffic of normal lymphocytes. It also plays a role in the metastatic spread of lymphoid malignancies.[19] In a study of mouse lymphomas, HEV-binding ability, as measured on frozen sections of lymph nodes or Peyer's patches, accurately predicted the tendency of lymphomas to spread hematogenously to HEV-bearing organs on passage into syngeneic recipients.[20] Lymphomas incapable of binding HEV *ex vivo* produced large local masses at the site of injection (eg, in the thigh), and invaded the blood and infiltrated the spleen and bone marrow, but failed to involve Peyer's patches or distant lymph nodes not draining the tumor. Lymphomas that bound well to HEV, on the other hand, characteristically spread early to lymph nodes and Peyer's patches, producing striking generalized lymphadenopathy. These experiments confirm that normal lymphocyte homing mechanisms can play a role in the *in vivo* behavior of lymphoid malignancies.

Molecules Involved in Lymphocyte–HEV Interactions

Vascular Addressins

The ability of lymphocytes to discriminate between HEV in different tissues implies that HEV must express tissue-specific determinants for lymphocyte recognition. Such vascular addressins, so called because of their role in providing tissue position or "address" signals to circulating lymphocytes, have now been identified in PLN and in mucosal lymphoid tissues (Table 1). The mucosal vascular addressin (MAd), defined by monoclonal antibodies MECA-367 and MECA-89, is selectively expressed by vessels involved in lymphocyte traffic into mucosal tissues,[21] including Peyer's patches, the mesenteric lymph nodes, the lamina propria, the small and large intestines, and the mammary gland. MECA-367 blocks lymph node lymphocyte binding to mucosal HEV and prevents lymphocyte extravasation into Peyer's patches almost completely. Interestingly, the antibody inhibits extravasation of gut-homing lymphoblasts into lamina propria, or into organized Peyer's patches, by only approximately 50% (P. Streeter, unpublished data). This observation suggests that additional mechanisms may be important in the traffic of activated mucosa-specific lymphocyte populations, perhaps a lamina propria-specific addressin or an inflammation-associated adhesion signal, as discussed above; or specific chemoattractants that may act on cells localized in the mucosal vasculature.[22]

Table 1. *Mouse and Human Molecules Involved in Lymphocyte-HEV Interactions*

	~Mr	Species	Inhibitory Ab	Effect on binding to HEV			Refs
				PLN	Mucosal	Synovial	
Vascular Antigens							
Mucosal Addressin	58-66 kd	Mouse	MECA-367	—	↓↓↓	(−)*	21
PLN Addressin	90, 110, others	Mouse and human	MECA-79	↓↓	—	(−)	25
Lymphocyte Antigens							
PLN homing rec.	80-100 kd	Mouse	MEL-14	↓↓↓	—	—	27
(LAM-1, ?HEBFLN,	75-85 kd	Human	DREG-55, -56	↓↓↓	—		28,46
Leu-8)			DREG-200	↓	—		
H-CAM (CD44,	85-95 kd	Human	Hermes-3	—	↓↓↓	—	7,32
Hermes antigen,			polyclonal	↓↓↓	↓↓↓	↓↓↓	
Pgp-1, ECMRIII, p80)							
LPAM-1	150/130 kd	Mouse	R1-2	—	↓↓↓		43
(VLA-4, CDw49d)		Human	Various				
LFA-1	150/95 kd	Mouse and human	Various	↓	↓		44,45
(CD11b/CD18)							
			(antibodies also inhibit monocyte and neutrophil-endothelial cell interactions)				

* Functional studies with mucosal and PLN-specific lymphoid cell lines indicate that the PLN and mucosal addressins are not the principal addressins involved in lymphocyte binding to venules in inflamed joints.[9] In addition, only a subset of synovial HEV express the PLN addressin, identified immunohistologically by MECA-79.

MAd is a 58–66 kD O-glycosylated glycoprotein[21] (E. Berg, unpublished data). It is an adhesion molecule for lymphocytes: isolated MAd, incorporated into artificial planar lipid membranes, binds lymphocytes avidly.[24] This binding exhibits the same cell specificity and antibody-inhibition characteristics as lymphocyte binding to Peyer's patch HEV.

The PLN addressin (PNAd), defined by MAb MECA-79 in both mice and humans, is preferentially expressed by HEV in PLN.[25] (It is also variably present at lower levels on HEV in mouse Peyer's patches and in human appendix, but in these mucosal sites binding *via* MAd predominates.) MECA-79 inhibits normal lymphocyte binding to PLN–HEV, but not to HEV in Peyer's patches, and it selectively inhibits lymphocyte homing to lymph nodes *in vivo.* Although the addressins have not been detected immunohistologically in acute inflammation or during the initial phase of mononuclear cell recruitment in subacute inflammation, PNAd is expressed at sites of long-standing chronic inflammation, especially, but not exclusively, when lymphocyte infiltration is dominant (L. Picker, P. Streeter, unpublished data). For example, the antigen is variably displayed by dermal venules in inflammatory dermatoses in humans, and appears at day 2 or 3 in human delayed-type hypersensitivity reactions. In mice, MECA-79 reactivity has not been observed in dermal inflammation, but PNAd does appear with MAd in the chronically inflamed pancreas in nonobese diabetic mice (P. Streeter et al, unpublished observation). PNAd is also expressed by a subset of vessels in lactating mammary glands. Thus PNAd can participate in lymphocyte localization to sites of chronic inflammation but, at least as defined by MECA-79, does not appear to be responsible for initiation of inflammation or for early mononuclear cell recruitment.

The MECA-79 antigen is detected in Westerns or immunoprecipitates as a lymph node-specific but heterogeneous set of glycoproteins (E. Berg, manuscript in preparation). The predominant species are O- and N-glycosylated glycoproteins of approximately 90 and 100 kD, but additional bands are recognized at 60, 70, 150, and 180 kD. Indeed, preliminary studies suggest that the PLN ligand for lymphocytes may be determined, in part, by a lymph node-specific post-translational modification, recognized by MECA-79, that decorates more than one glycoprotein on PLN HEV.

What determines the local differentiation of HEV and the tissue-specific expression of vascular addressins? It seems likely that unique microenvironmental factors control HEV differentiation *in vivo,* perhaps involving a combination of conventional immune cytokines with tissue-specific signals. In this context, it is interesting that mesenteric lymph nodes, unique in that they drain a mucosal tissue, the intestines, are also the only lymph nodes in adult mice whose HEV express high levels of PNAd and MAd simultaneously. This suggests that cellular or humoral factors derived from the intestines and arriving in lymph can influence the patterns of addressin expression in mesenteric node HEV. High endothelial differentiation and addressin expression appear to be independent of lymphocyte traffic or stimulation: normal levels and specificities of addressins are expressed in lymphoid tissues in germ-free mice and in SCID mice that are essentially devoid of lymphocytes (P. Streeter, unpublished observations).

Interestingly, at birth vessels in mouse PLN express high levels of the mucosal vascular addressin, and in contrast lack significant PNAd (P. Streeter, unpublished observation). PNAd begins to be expressed within 1 to 2 days of birth and increases rapidly to adult levels by about 7 to 10 days. MAd gradually decreases in intensity in PLNs over 4 weeks. The adult pattern is only reached at 4 to 6 weeks of age. These developmental changes in addressin expression have been confirmed in functional assays using PLN or mucosal HEV-specific lymphoid cell lines. MAd is also expressed before birth on most vessels in the developing intestines. The widespread expression of MAd in the late fetal and postnatal period may reflect ontogeny recapitulating phylogeny, cut could also be physiologically significant. There is considerable evidence[26] that maternal lymphocytes in milk can transfer cellular immune responses to suckling infants (eg, delayed-type hypersensitivity and even, in some circumstances, graft-*versus*-host reactions leading to runting). Because lymphocyte homing to the mammary gland involves, at least in part, mucosa-specific mechanisms[2] (personal observations of partial inhibition of homing of mesenteric node blasts to mammary glands by MECA-367), widespread expression of the mucosal addressin in infants could facilitate the participation of such maternal lymphocytes in immune responses in their adoptive hosts.

Lymphocyte Homing Receptors for HEV

Antibody inhibition studies have led to the identification of several lymphocyte surface molecules involved in adhesion to HEV (Table 1). These include the PLN homing receptor, defined originally by MEL-14 in the mouse,[27] and now identified by a number of monoclonal antibodies in humans, including DREG-55, -56, and -200,[28] LAM-1,[29] and Leu-8 (T. Tedder, oral personal communication, October 1989). MEL-14 and the DREG-55 and -56 antibodies inhibit lymphocyte binding to PLN HEV, but not to mucosal or synovial HEV, and recognize an 80 to 90 kD mouse and 70 to 80 kD human glycoprotein that is expressed on PLN–HEV binding but not Peyer's patch HEV-specific or nonbinding lymphocytes. Rosen, Stoolman, and colleagues[14] have presented convincing evidence that the PLN homing receptor is a mammalian lectin that can bind to PLN–HEV *via* a carbohydrate-inhibitable mechanism. In our hands, the ability of antibodies to the human PLN homing receptor to inhibit HEV binding correlates well with their ability to block homing-receptor binding of the phosphomannan PPME.[28] The intimate link between the lectin and HEV-binding activities of the PLN homing receptor suggests that the HEV ligand for this receptor will prove to contain a critical carbohydrate element. Strong support for this comes from the demonstration that sialidase treatment of peripheral node (but not Peyer's patch) HEV abrogates their ability to bind lymphocytes.[14] In addition, the cDNA sequence of PLN homing receptors[29–31] shows an N-terminal domain homologous to C-type lectins (eg, the asialoglycoprotein receptor) but containing an unusually high frequency of basic residues, perhaps relating to the acidic nature of inhibitory sugars.[31] An additional sequence homologous to EGF, and two repeating units related to sequences in complement regulatory proteins, raise the possibility that PLN homing-receptor interaction with its HEV ligands involves multiple molecular domains.[30]

H-CAM (*h*oming-*a*ssociated *c*ell-*a*dhesion *m*olecule), which we have previously referred to as the Hermes antigen, represents another class of homing receptor that can apparently participate in all lymphocyte–HEV interactions. Its role in lymphocyte–HEV recognition, like that of the PLN homing receptor, was defined originally by antibody inhibition studies.[7,32] For example, MAb Hermes-3 blocks human lymphocyte binding to mucosal but not to PLN–HEV, and polyclonal antisera to H-CAM block lymphocyte binding to all HEV classes (including synovial HEV). Collaborative studies have recently shows that H-CAM is CD44, ECMRIII, P80, and Pgp-1.[33–35] As Pgp-1, H-CAM is known to be expressed at elevated levels by memory lymphocytes, especially in certain strains of mice,[17] but also in humans[18] and primates (W.M. Gallatin, personal communication). As ECMRIII, it has been shown to interact with extracellular matrix components including collagen (reviewed in Gallatin et al[33]) and Jalkanen and coworkers also have shown that lymphocyte H-CAM binds to collagen and to the heparin-sulfate binding domain of fibronectin (S. Jalkanen, personal communication).

Immunohistologic studies of non-Hodgkin's lymphomas and leukemias suggest that H-CAM expression may facilitate but itself is not sufficient to predict hematogenous lymph node metastasis.[36] A panel of antibodies against diverse homing receptors may allow more precise predictions of homing patterns of lymphomas.

We and others have recently cloned H-CAM.[37–39] The cDNA sequence reveals an N-terminal sequence homologous to a globular domain present in cartilage link protein and in cartilage and other proteoglycan core proteins. This domain is thought to support link protein interaction with hyaluronic acid and protein components of cartilage. In H-CAM, it may interact with glycosaminoglycan or glycoprotein elements on HEV. This N-terminal sequence is highly conserved in mouse and human H-CAM (89% identity).[35] A proximal extracellular domain, in contrast, is only 40% identical between the two species. This latter domain lacks cysteines and contains abundant utilized O-glycosylation sites, as well as four potential chondroitin sulfate-linkage sites. (Interestingly, a subset of lymphocyte H-CAM is modified by covalent linkage to chondroitin

sulfate: H-CAM can therefore be regarded as a facultative proteoglycan.[40]) It is possible that the function of this domain is determined, in large part, by its carbohydrate modifications. Nonetheless, this domain may be directly important in some functions of H-CAM: most monoclonal antibodies against H-CAM appear to recognize epitopes in this stalk region, including MAb Hermes-3, which inhibits lymphocyte binding to mucosal HEV.[36]

Inhibition of lymphocyte binding to mucosal HEV by MAb Hermes-3 raised the possibility that H-CAM is a lymphocyte receptor for MAd. Fluorescent energy-transfer/quenching studies using isolated MAd and H-CAM confirm that these molecules bind each other in solution in a saturable, reversible manner[41] (M. Nakache, personal communication). This binding is inhibited by Hermes-3 and by anti-MAd MAbs. Thus H-CAM appears to be one lymphocyte surface receptor for MAd. Whether it can also interact with PNAd or other HEV elements remains to be determined.

H-CAM is clearly more than just a homing receptor, however. It is a widespread molecule expressed by diverse cell types in the body (reviewed in Picker et al[41]), both in the adult and in the developing embryo. For example, it displays fascinating developmental and site-specific regulation on glial cells during neurogenesis and is likely to play an important role, along with other neural and glial cell adhesion molecules, in the histogenesis of the nervous system (H. Vogel, L. Picker, manuscript in preparation). In addition to interactions with HEV and with extracellular matrix components, H-CAM has been implicated in T-cell adhesive interactions involving LFA-3 and CD2 (reviewed in Haynes et al[42]). On most cell types, as on lymphocytes, H-CAM is heterogeneous in size but displays a predominant Mr of approximately 90 kD.[41] Certain epithelial cells, however, express a unique form of the molecule of Mr approximately 150 kD that is apparently encoded by mRNAs of unique size as well.[37]

In addition to these novel classes of adhesion molecules, at least two members of the integrin family also are implicated in lymphocyte–HEV interaction. Holzmann and Weissman[43] have shown that antibodies to LPAM-1, a mouse integrin, block lymphocyte binding to Peyer's patch HEV. The α chain of LPAM-1 appears to be the mouse homologue of human α4(VLA-4α, CDw49d), and in preliminary experiments anti-α4 antibodies inhibited Peyer's patch-specific binding of human lymphocytes, as well (B. Holzman and N. Wu, unpublished observations). Like H-CAM, LPAM-1 (or at least its α chain) display a broad tissue distribution. Peyer's patch-HEV specificity may be determined by association of α4 with a unique β chain[43]; or LPAM-1 may be another part-time homing receptor. It will be important to determine if LPAM-1, like H-CAM, is a receptor for MAd.

Another integrin, LFA-1 (CD11a/CD18), can also participate in lymphocyte–HEV interactions, although it is not required: antibodies to LFA-1 partially inhibit lymphocyte binding to both lymph node and mucosal HEV[44,45] (N. Wu, unpublished data), but B lymphoblastoid cell lines from patients with leukocyte adhesion deficiency, a genetic absence of the LFA-1 β chain (CD18), can bind well to HEV (N. Wu, unpublished data).

Several other molecules have also been implicated as lymphocyte homing receptors. Woodruff, Chin, and colleagues,[46] by identifying high endothelial-binding factors (HEBF) on lymphocytes or from lymph or serum, have defined PLN homing receptors in rats and in humans and an apparently unique 80-kD Peyer's patch homing receptor in rats. It would be very valuable to compare these with the homing receptors mentioned above. Finally, antibodies to a 30-kD guinea pig lymphocyte antigen also inhibit lymphocyte–HEV binding.[47] The functional assays discussed above suggest that several additional lymphocyte adhesion molecules/homing receptors, and their vascular ligands, remain to be identified.

Regulation of Neutrophil and Monocyte Extravasation

Neutrophils, monocytes, natural killer cells, and eosinophils express PLN homing receptors. Indeed, MEL-14 blocks neutrophil binding to PLN HEV *ex vivo* (but not to Peyer's patch HEV, indicating that neutrophils, like lymphocytes, use distinct mechanisms to bind mucosal vessels); and MEL-14 inhibits neutrophil localization to extralymphoid sites of acute inflammation *in vivo*, including the dermis or peritoneum.[48,49] Neutrophils and monocytes also express high levels of H-CAM.

If these leukocytes express the same homing receptors as lymphocytes, how can we explain the fact that they do not behave like lymphocytes? For example, why don't neutrophils recirculate constitutively through PLN and Peyer's patches? Recent studies suggest that there must be considerable sophistication in the functional regulation of known adhesion molecules, and that additional leukocyte-specific adhesion mechanisms must also exist.

Inflammation Induces Endothelial Cell Adhesion Molecules for Neutrophils and Monocytes

At the cellular level, it is now clear that the extravasation of neutrophils, monocytes, and lymphocytes during inflammation is controlled, in part, by the induction of leukocyte-specific adhesion mechanisms on EC. In the *ex vivo* frozen-section assay, monocytes and monocytoid cell lines fail to bind to HEV in inflamed lymph nodes, but bind very well to HEV in lymph nodes three days after stimulation by footpad injection of complete Freund's adjuvant.[49]

The induced change in HEV parallels precisely the observed association of circulating monocytes *in vivo* with vessels in the same lymph nodes, assessed immunohistologically. The change is monocyte specific in that the constitutive binding of neutrophils and lymphocytes to HEV is unaltered during the course of the inflammation. Surprisingly, even though monocytes fail to bind to HEV in control nodes, binding to inflamed HEV involves the PLN homing receptor (the MEL-14 antigen). Only MEL-14^+ and not MEL-14^- monocytoid cell lines bind, and binding is inhibited by MEL-14. MEL-14 also inhibits monocyte extravasation into thioglycolate-inflamed peritoneum, supporting a general role for the PLN homing receptor in leukocyte-EC interactions during inflammation (at least in nonmucosal sites). The leukocyte integrin Mac-1 is also involved in these interactions (reviewed in Jutila et al[49]): anti-Mac-1 antibodies inhibit monocyte interactions with HEV *ex vivo* and monocyte extravasation into inflamed tissues *in vivo*.

Neutrophil extravasation is also regulated, in part, by induced EC adhesiveness. Injection of LPS intradermally leads to a dramatic increase in the ability of local venules in frozen sections to bind applied neutrophils, and the kinetics of increased adhesiveness for neutrophils parallels the observed influx of neutrophils into the tissues *in vivo,* occurring over 2 to 3 hours (*versus* 1 to 3 days for the induction of monocyte adhesiveness in HEV) (M. Jutila, E. Berg, personal communication). These observations strongly support the existence and precise regulation of neutrophil- and monocyte-specific adhesion molecules on EC.

In vitro studies of human umbilical vein endothelial cells (HUVEC) have also shown convincingly that EC can be induced by LPS or by cytokines (IL-1 or TNF-α) to express increased adhesiveness for leukocytes (reviewed in Cotran[50]). Interestingly, one induced adhesion molecule for neutrophils, ELAM-1, proves to be closely related structurally to the PLN homing receptor, displaying homologous lectin, EGF, and repeating complement regulatory protein-related units.[51] Another member of this novel gene family is GMP-140 (CD62), a platelet and EC antigen that is rapidly translocated to the cell surface from intracellular pools by cellular activation.[52] GMP-140 may also participate in neutrophil-EC interactions (R. McEver, personal communication).

Neutrophil binding to IL-1 or TNF-stimulated HUVEC involves both Mac-1 and the PLN homing receptor, supporting the relevance of this *in vitro* system to the physiologic situation (R. Hallmann, M. Jutila, K. Kishimoto, W. Smith, manuscript submitted for publication). There is evidence that yet a third adhesion mechanism participates in this interaction. Interestingly, lymphocyte binding to such stimulated HUVEC, unlike neutrophil binding, is not inhibited by DREG-56 (R. Hallmann, unpublished observation). Furthermore, neither IL-1, TNF, nor other cytokines tested have induced expression of PNAd on HUVEC. This suggests that neutrophil and lymphocyte PLN homing receptors can bind distinct ligands, and that (as suggested by immunohistologic and *ex vivo* studies of human inflammatory sites), cytokines associated with inflammation induce EC to bind lymphocytes by mechanisms distinct from those involved in lymphocyte homing to organized lymphoid tissues. It will be important in the future to determine the EC and leukocyte ligands for the known molecules involved, and to identify the additional leukocyte- and lymphocyte-specific adhesion molecules induced during inflammation.

Circulating Leukocytes Can Respond to Inflammatory Signals

Leukocytes may also play an active role in adhesion, responding to inflammatory stimuli while still in the vascular lumen. Morphologic observations of membrane ruffing in marginating neutrophils, as well as the phenomenon of neutrophil–neutrophil aggregation in inflamed vessels, have been used to argue that neutrophils respond actively to the inflammatory microenvironment while still in the vascular compartment.

We found that mouse and human neutrophils activated *in vivo* by inflammation or *in vitro* by phorbol esters or by chemoattractants such as leukotriene B4 or complement component C5a respond rapidly by shedding their surface PLN homing receptor (an approximately 50% decrease in one minute; nearly 100% shed by four minutes), probably by proteolytic cleavage near the plasma membrane.[53,54] With the same kinetics, the level of neutrophil surface Mac-1 increases approximately fivefold. The kinetics of these alterations would permit them to occur during rolling of neutrophils along inflamed venules, as observed in sites of inflammation or tissue damage; and in fact, in immunohistologic studies many neutrophils inside vessels are MEL-14^+ and Mac-1^{dull}, like isolated peripheral blood neutrophils, but most neutrophils within the vascular wall and all within the surrounding tissues are MEL-14^- and Mac-1^{bright}.[54] In combination with the observation that anti-CD18 (anti-Mac-1 β chain) MAbs prevent irreversible arrest of neutrophils but not initial EC attachment and rolling,[55] this has led to the hypothesis[49] that the PLN homing receptor is involved in early binding to the EC, perhaps slowing neutrophil transit and permitting them to respond to activating factors entering the venule (or displayed at the EC surface). Neutrophil activation then rapidly enhances Mac-1 function and expression, at least in part due to exocytosis of neutral granules, cementing neutrophil adhesion. Mac-1 probably also par-

ticipates in subsequent diapedesis and chemotaxis. Simultaneously, the PLN homing receptor is shed. This may serve to facilitate release of neutrophils from EC after transmural migration or may help prevent ectopic EC attachment of activated neutrophils released into the circulation. Whether similar (if less dramatic) activation occurs during monocyte and lymphocyte-EC interactions in inflamed tissues remains to be determined. The PLN homing receptor is also shed from monocytes and lymphocytes activated with phorbol esters, albeit more slowly than from neutrophils.

Thus leukocyte-EC interactions are regulated at multiple levels, involving inflammation-induced expression of adhesion molecules on EC, and rapid activation-linked alterations in the adhesive properties of circulating leukocytes (at least neutrophils).

Concluding Remarks

Leukocyte-EC interactions clearly represent a complex and fascinating model of cellular recognition and adhesion. Identification of the many leukocyte and EC adhesion molecules involved will only be the first, and perhaps the easiest step in our efforts to define the mechanisms controlling these interactions. Complete understanding will require not only reconstitution of HEV-binding behaviors in model cells through recombinant DNA technology, but also careful attention to the relevance of such *in vitro* studies to the physiologic situation *in vivo*. On the positive side, the involvement of several adhesion molecules offers multiple targets for molecular approaches to regulating or manipulating particular leukocyte-EC interactions, a worthy goal given that such interactions represent the first essential step in all inflammatory and immune responses. Intervention at the level of leukocyte-EC adhesion may eventually permit tissue-specific immunosuppression, for example in rheumatoid arthritis, or leukocyte or lymphocyte-subset-specific inhibition of trafficking, allowing manipulation of the nature of local inflammatory responses.

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